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In Re U.S. Patent Application		
Applicant(s):	*Eberhardt, et al.*	
Serial No.:	10/821,074	*I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Arlington, VA 22313-1450*
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For:	FAST MULTI-LINE LASER CONFOCAL SCANNING MICROSCOPE	06/15/2004
Attorney Docket No.:	9345/91883	Date Signature

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Respectfully submitted,

By:
Jaffrey W. Salmon, Esq.
Registration No. 37,435

Dated: June 15, 2004

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Registration No. 37,435

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference — 13188/ME/st

2. Patent application number *(The Patent Office will fill in this part)* — 0308072.8 — 08 APR 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

   VisiTech International Ltd
   Sunderland SR5 2TQ
   United Kingdom

   Patents ADP number *(if you know it)* — 7926074002

   If the applicant is a corporate body, give the country/state of its incorporation — England

4. Title of the invention — Fast multi-line laser confocal scanning microscope

5. Name of your agent *(if you have one)* — Henkel, Feiler & Hänzel- Patent Attorneys Möhlstrasse 37 - D 81675 München 6657845001

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

   Ken Bell
   VisiTech International Ltd
   Unit 92, Silverbriar
   Sunderland Enterprise Park (East)
   Sunderland SR5 2TQ, UK

   Patents ADP number *(if you know it)* — 8605479001

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* — yes
   a) any applicant named in part 3 is not an inventor, or
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   c) any named applicant is a corporate body.
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Claim *(s)*	7
Abstract	1
Drawing *(s)*	3 + 3

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	21
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Patentanwälte
Henkel, Feiler & Hänzel
Möhlstr. 37 • D-81675 München

Signature
M. Garkisch

Date
April 7, 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

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13188

VisiTech International Ltd

Fast multi-line laser confocal scanning microscope

Description of the Prior Art

The inclusion of an acousto-optical deflector to produce the line scanning of a laser spot in a laser confocal microscope creates a unit which can operate with high speed and flexibility, i.e. with variable scanning amplitude, and suitable for various types of confocal microscopy. As a result of rapid line and frame scanning, it is then possible, in a very short space of time, to combine electronically a number of thin image sections to form an image with an increased depth of focus. Such collections of image sections are readily converted into a 3-Dimensional reconstruction of the original object volume, whereby spatial relationships between the object components can be rapidly visualised and measured.

Figure 1 indicates an example of a laser scanning confocal microscope in which an acousto-optical deflector is used to provide the line scanning of a laser spot. Such confocal laser scanning microscope is disclosed in EP-A-0 284 136 and its content is herewith included by reference.
A laser light beam 1 passes a beam expansion optical system 2 and 3, followed by a beam splitter 4, an acousto-optical deflector 5 having a planocylindrical lens 5.1 and a plano convex lens 5.2 both at the entrance and at the exit side, a lens 6, a deflector 7, which may be a mirror galvanometer, a lens 8, a quarter wave plate 16, and an objective 9 for focussing the laser beam 18 onto an object. In an object plane 10, an object, not shown, is further placed on a stationary object stage. The reflected light 19 traverses a return path identical to the outward path up to the beam splitter 4 after which it is split off to a polarising filter

11, a further objective 13, a spatial filter 14, a lens 17, a band pass or cut-off filter 12, and finally a detector 15.

Figure 2 indicates another example. A dichromatic mirror 20 has been incorporated in the light path between the planocylindrical lens 5.1 and the lens 6. Said mirror transmits the (short wave) laser light and deflects the long-wave return light originating, for example, from fluorescence. Note that a simple change in geometry will permit the use of a dichromatic mirror that reflects the (short wave) laser light and transmits the long-wave returm light. This light is passed through a correction lens 21 and focussed with an objective 22 on a spatial filter 23 which is a slit filter, as a result of which this system has confocal characteristics. In this manner, a line detector is formed with a subsequent lens 24 and a detector 26. Between the lens 24 and the detector 26 one or more band pass or cut-off filters 25 has been incorporated which has the same function as that of the band pass or cut-off filter 12. With this embodiment, return light that has a wavelength other than that of the outward light can be advantageously examined if the acousto-optical deflector has too low an efficiency for said light, i.e. brings too large an attenuation.

Background of the Invention

In biological objects that are autofluorescent, or have been labelled with fluorescent probes, a frequent requirement is to extract multi-spectral component images from the object that permit the spatial relationships between the variously labelled components to be studied. Living biological objects contain dynamic processes that may be fluorescently labelled so that, by repeating the same multi-spectral scan of the object, the temporal dynamics of these processes may also be studied. In all of these types of experiments it is important to have the ability to rapidly switch excitation wavelengths during the scanning process and the invention aims at providing a method of achieving fast multi-wavelength

scanning in an acousto-optical deflector based laser confocal scanning microscope providing fast multi-wavelength scanning.

Summary of the Invention

The present invention provides a laser confocal scanning microscope as defined in claim 1 or in claim 2 and a method of achieving fast multi-wavelength scanning in an acousto-optical deflector based laser confocal scanning microscope as defined in claim 18 or 19. Preferred embodiments are defined in the dependent claims.

Brief description of the drawings

Figure 1 indicates an example of a laser scanning confocal microscope in the prior art in which an acousto-optical deflector is used to provide the line scanning of a laser spot;

Figure 2 indicates another example of a laser scanning confocal microscope in the prior art;

Figure 3 is a diagram explaining the range of usable deflection angles available from an Acousto-Optical-Deflector (AOD) used in a laser scanning confocal microscope in accordance with the present invention.

Description of preferred embodiments

When an acousto-optical deflector is used as a deflection element in a scanning system there are a number of disadvantages that need to be considered.

As a result of the dispersive nature of the acousto-optical deflector, the return light of a wavelength (for example, fluorescence) other than the laser light no longer passes through the spatial filter. The spatial filter may be displaced by three piezo electric crystals, one for each of the three axes of the XYZ co-ordinate system and controlled in a manner that eliminates this effect.

Due to the time of transit across an acousto-optical deflector aperture of a change in the acoustic wave frequency, an acousto-optical deflector produces a cylindrical 'lensing' effect caused by the difference in deflection angles of the incident wavelength at the two ends of the acousto-optical deflector aperture. The difference in deflection angle between the ends of the acousto-optical deflector aperture increases with increased deflection scanning rates since the time of transit across the acousto-optical deflector aperture is constant.

The Bragg equation (wavelength = 2 x acoustic frequency x sin(deflection angle)) describes how the deflection angle is dependent on the wavelength of a light beam passing through an acousto-optical deflector. The absolute deflection angle and the difference in deflection angles of the light beam at the ends of the acousto-optical deflector aperture increase with increasing wavelength of the light beam when an oscillator driving the acousto-optical deflector follows a constant repetitive pattern of frequency sweeps.

Without attempts to correct for the lensing effects described above, changes in the wavelength of the illuminating light beam and changes in the rate of scanning will effect the position and size of the scanned area on the sample and also degrade the image quality due to the introduction of astigmatism in the optical path. The invention offers two methods of compensation for the lensing effect.

According to a first embodiment a method of achieving fast multi-wavelength scanning in an acousto-optical deflector based laser confocal scanning microscope according to the invention comprises dynamically adjusting an optical path of said an acousto-optical deflector based confocal microscope by mechanical means in accordance with a selected wavelength of a laser light beam, to compensate for astigmatism and collimation changes due to the change in input beam

wavelength and modifying detected images of an object by electronic means to maintain alignment of the scan lines of the image at all wavelengths.

A laser confocal scanning microscope according to this embodiment of the invention comprises: means, including a laser light source, for emitting laser light beams at different wavelengths; a beam path for directing said laser light beams from said laser light beam emitting means to an object stage for supporting an object, said beam path including a first deflector including an acousto-optical deflector for effecting line scanning, at least one objective for focussing the laser light beams onto the object on said object stage, a second deflector positioned between said acousto-optical deflector and said at least one objective, for effecting frame scanning, said second deflector and said at least one objective being positioned so that return light beams from the object follow the same beam path as the laser light beams focussed onto the object up to and including the second deflector, at least one detector positioned in the return light beam path downstream said second deflector, for detecting the return light beams from the object, the object being adapted to be scanned by the laser light beams from the laser light beam emitting means and measurements being adapted to be made with said at least one detector in order to obtain images of the object, and an electronic control and imaging system adapted to control the laser light beam emitting means to emit laser light beams of different selected wavelengths and adapted to dynamically adjust the optical path by mechanical means in accordance with the selected wavelength of the laser light beams, to compensate for astigmatism and collimation changes due to the change in input beam wavelength and adapted to modify the obtained images of the object by electronic means to maintain alignment of the scan lines of the image at all wavelengths.

A first embodiment thus describes compensation for the astigmatism and focus effects previously described using mechanical means to change the position of correction optical components within the optical path. An astigmatism lens is moved in position to correct for astigmatism changes and a collimating lens is moved to ensure that the beam entering a final objective is focussed by that objective to the same focal plane in the object being scanned. Remaining changes in the position of the image caused by the changes in scan line position on the object, due to deflection angle changes with wavelength changes, are compensated by using software pan, zoom and clipping of the recorded image data to maintain identical image size and position for all wavelengths.

Correction of the astigmatism and collimation by mechanical means is often too slow to enable the rapid switching of input beam wavelengths desired in biological research. For the study of dynamics in biological objects it is preferable to have wavelength switching on a scan line by scan line basis with scan line frequencies typically in the tens of kilohertz range.

According to a second embodiment a method of achieving fast multi-wavelength scanning in an acousto-optical deflector based laser confocal scanning microscope according to the present invention comprises dynamically adjusting an optical path of said an acousto-optical deflector based confocal microscope by mechanical means in accordance with a selected wavelength of a laser light beam, to compensate for astigmatism and collimation changes due to the change in input beam wavelength and modifying detected images of an object by electronic means to maintain alignment of the scan lines of the image at all wavelengths.

A laser confocal scanning microscope according to this embodiment of the invention comprises: means, including a laser light source, for emitting laser light beams at

different wavelengths; a beam path for directing said laser light beams from said laser light beam emitting means to an object stage for supporting an object, said beam path including a first deflector including an acousto-optical deflector for effecting line scanning, at least one objective for focussing the laser light beams onto the object on said object stage, a second deflector positioned between said acousto-optical deflector and said at least one objective, for effecting frame scanning, said second deflector and said at least one objective being positioned so that return light beams from the object follow the same beam path as the laser light beams focussed onto the object up to and including the second deflector, at least one detector positioned in the return light beam path downstream said second deflector, for detecting the return light beams from the object, the object being adapted to be scanned by the laser light beams from the laser light beam emitting means and measurements being adapted to be made with said at least one detector in order to obtain images of the object, and an electronic control and imaging system adapted to control the laser light beam emitting means to emit laser light beams of different selected wavelengths and adapted to dynamically adjust drive parameters of said acousto-optical deflector in accordance with the selected wavelength of the laser light beams, to maintain alignment of the scan lines of the image at all wavelengths.

A second embodiment thus describes a method of achieving fast corrections for the lensing effect in an acousto-optical deflector enabling rapid switching of input beam wavelengths such that successive scans along a line in the object may be made at different wavelengths without reducing the scanning frequency.

In a first embodiment mechanical means are used to move an astigmatism and a collimating lens to predetermined positions for each input beam wavelength by an electronic control

system. Such mechanical means may be provided by electrical motors of various types, including AC, DC and stepper types, or by electromechanical actuators such as piezoelectric crystals. The position of the astigmatism lens has an almost linear relationship with the wavelength of the input beam as it corrects for the change in deflection angles due to changes in the wavelength of the input beam. The position of the collimating lens is determined experimentally for each wavelength as this lens compensates for any changes in focus from the changes in position of the compensating optical elements.

The electronic control system may also calculate control signals suitable for synchronising the selection of an input beam wavelength and also for synchronising an intensity modulation and/or blanking of the input beam during any chosen portion of the scanning pattern. Positional changes in the image are corrected by pan, zoom and clipping of recorded image data by software in an associated imaging system.

In a second embodiment an electronic control system provides dynamic control of the start frequency, end frequency and rate of frequency change of the drive signal to the acousto-optical deflector in such a way as to maintain constant line scanning position and a constant lensing effect at all input beam wavelengths.

From the Bragg equation it is known that to maintain the same deflection angle for any chosen input beam wavelength the acoustic grating spacing must change proportionally, this is effected by changing the frequency of an oscillator that drives the acousto-optical deflector. The drive oscillator frequency is swept over a range of values to effect the scanning of the laser beam along a line. Adjusting the range of the sweep frequencies in accordance with the Bragg equation for each input beam wavelength enables an identical

line of spot positions to be scanned at any of these wavelengths.

The drive oscillator start and end frequencies are calculated from the known deflection characteristics of the acousto-optical deflector for the input beam wavelengths and are chosen dynamically to maintain an identical optical scan at each wavelength of the input beam, and hence maintain a constant lensing effect at each input beam wavelength. Thus the optical system may be optimised for one lensing condition, this condition being maintained dynamically for all scan rates and input beam wavelengths through software computer control of the acousto-optical deflector drive parameters.

To maintain an identical scan rate over identically positioned pixels in the scanned object for any input beam wavelength requires that the deflection angles of an acousto optical deflector at the start and end of each scan should be the same at all input beam wavelengths.
From the Bragg equation it is obvious that the deflection angle of the light passing through an acousto optical deflector increases directly in proportion to the wavelength of that light beam when the acoustic drive frequency and hence the acoustic wavelength are held constant.

If we define the maximum acoustic frequency available to drive the Acousto-Optical Deflector (AOD) as providing the maximum deflection (corresponding to one, say right, edge of the scanned area) for the shortest light wavelength that must be deflected, and likewise define the minimum acoustic frequency available to drive the AOD as providing the minimum deflection (corresponding to the other, say left, edge of the scanned area) for the longest light wavelength that must be deflected, then any range of scanned line lengths, between the said left and right edges of the scanned area, can be duplicated at these or any intermediate wavelength. The

acoustic frequencies necessary to provide a required scan line length and position within the said range at any other required wavelength can be calculated in proportion to the wavelength of the light to be deflected.

With reference to Figure 3, lambda1 is the longest light wavelength to be deflected and lambda2 is the shortest light wavelength to be deflected. f1 is the maximum acoustic frequency available from an AOD driver oscillator and f2 is the minimum acoustic frequency available from the AOD driver oscillator.

At any intermediate light wavelength to be deflected (lambdaN), the deflection angle for the right edge (at acoustic frequency f1) is proportional to lambdaN / lambda2, and the deflection angle for the left edge (at acoustic frequency f2) is proportional to lambdaN / lambda1.

The range of useable deflection angles common to the range of light wavelengths lies between deflection angles Φ1 and Φ2. Therefore, the acoustic frequency to drive a chosen light wavelength lambdaN to the left edge is proportional to the ratio of
(maximum wavelength lambda1 / chosen wavelength lambdaN). Likewise, the acoustic frequency to drive a chosen light wavelength to the right edge is proportional to the ratio of (minimum wavelength lambda2) / (chosen wavelength lambdaN).

Knowledge of the input control voltage to acoustic drive frequency transfer characteristics of the drive oscillator for the AOD allows the electronic processor to calculate and output the scan control voltages necessary to create the required frequency sweeps that control the light beam deflection in the AOD. The electronic processor may also calculate control signals suitable for synchronising the selection of the input beam wavelength and also for

synchronising the modulation or blanking of the input beam during any chosen portion of the scan waveform.

The invention offers the ability to control the position of the optical line scan very rapidly, enabling successive frame scans and even successive line scans to be made at different input beam wavelengths. This permits fast multi-colour scans to be made where input beam wavelengths are switched sequentially on repetitive scans of the same scan line and then repeating the same, or a different, sequence of input beam wavelengths on the next and subsequent scan lines. For even faster views of the sample under multicolour input beam scanning, the input beam wavelengths may be switched sequentially for adjacent scan lines, however this additional increase in image capture speed is gained at the loss of spatial resolution and registration of the images at each input beam wavelength.

In an advantageous embodiment a programmable voltage ramp generator drives a control input of a voltage controlled radio frequency oscillator that drives the acousto-optical deflector. Thus, defining the start voltage, the end voltage and the rate of voltage change of (typically) a classical sawtooth waveform, the programmable voltage ramp generator provides the appropriate RF signal to the acousto-optical deflector to scan the laser beam across the object. These voltage parameters can be altered dynamically at any time so that deflection changes due to changes in input beam wavelength can be compensated in the shortest practical time, limited only by the propagation speed of the acoustic wave in the acousto-optical deflector and the dimensions of the acousto-optical deflector aperture. These changes may synchronise, or be synchronised by, external events such as switching of the input beam wavelength. For example, an acousto optical tuneable filter (AOTF) at the output of a multi-line laser can be synchronised with the flyback period of the sawtooth waveform. Using an AOTF also provides a

simple and efficient means of modulating or blanking of the laser intensity for the period of time during which the acousto-optical deflector drive signal is changing to a new scan condition, typically but not exclusively, during the flyback time at the commencement of a new scan line or a new scan frame. As acousto-optical deflectors (AOD's) also have the ability to modulate the intensity of the input light beam on its passage through the AOD, the AOD may also be used as a means to modulate and blank the input beam intensity.

Either of the two embodiments described may be further adapted downstream from the separation of the return beam path from the input beam path by the beamsplitter (4) or the dichromatic mirror (20), to divide the return light beam path by means of additional beam splitters, dichromatic mirrors or combinations of both. Each resulting beam is adapted to be directed to one of a plurality of detectors, each detector having a spatial filter, objective, and lens duplicating the single detector beam path shown in the Figures 1 and 2, except that a bandpass or cut-off filter may be different for each detector path, therefore this plurality of detectors may be used to extract spectral information from the return light beam.

Additional advantages of this invention may also include the following:
The scanned area of the object is identical for all wavelengths so there is no scanning of the object outside of the area of interest to cause photo-bleaching effects on adjacent areas of the object.
Pixels taken from the same object at different input beam wavelengths have identical positions and dwell times which simplifies the application of the confocal microscope to such techniques as FRET (Fluorescence Resonance Energy Transfer, FLIM (Fluorescence Lifetime Imaging), FRAP (Fluorescence Recovery After Photobleaching), image ratioing etc.

It is understood that the invention is not confined to the embodiments set forth herein as illustrative, but embraces such modified forms thereof as come within the scope of the following claims.

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Claims

1. A laser confocal scanning microscope comprising:

means, including a laser light source (1), for emitting laser light beams at different wavelengths;

a beam path for directing said laser light beams from said laser light beam emitting means to an object stage for supporting an object, said beam path including

a first deflector including an acousto-optical deflector (5) for effecting line scanning,

at least one objective (9) for focussing the laser light beams onto the object on said object stage,

a second deflector (7) positioned between said acousto-optical deflector (5) and said at least one objective (9), for effecting frame scanning,

said second deflector (7) and said at least one objective (9) being positioned so that return light beams (19) from the object follow the same beam path as the laser light beams focussed onto the object up to and including the second deflector (7),

at least one detector (15;26) positioned in the return light beam path downstream said second deflector (7), for detecting the return light beams from the object, the object being adapted to be scanned by the laser light beams from the laser light beam emitting means and measurements being adapted to be made with said at least one detector (15;26) in order to obtain images of the object, and

an electronic control and imaging system adapted to control the laser light beam emitting means to emit laser light beams of different selected wavelengths and adapted to dynamically adjust drive parameters of said acousto-optical deflector (5) in accordance with the selected wavelength of

the laser light beams, to maintain alignment of the scan lines of the image at all wavelengths.

2. A laser confocal scanning microscope comprising:

means, including a laser light source (1), for emitting laser light beams at different wavelengths;

a beam path for directing said laser light beams from said laser light beam emitting means to an object stage for supporting an object, said beam path including

a first deflector including an acousto-optical deflector (5) for effecting line scanning,

at least one objective (9) for focussing the laser light beams onto the object on said object stage,

a second deflector (7) positioned between said acousto-optical deflector (5) and said at least one objective (9), for effecting frame scanning,

said second deflector (7) and said at least one objective (9) being positioned so that return light beams (19) from the object follow the same beam path as the laser light beams focussed onto the object up to and including the second deflector (7),

at least one detector (15;26) positioned in the return light beam path downstream said second deflector (7), for detecting the return light beams from the object, the object being adapted to be scanned by the laser light beams from the laser light beam emitting means and measurements being adapted to be made with said at least one detector (15;26) in order to obtain images of the object, and

an electronic control and imaging system adapted to control the laser light beam emitting means to emit laser light beams of different selected wavelengths and adapted to dynamically adjust the optical path by mechanical means in accordance with the selected wavelength of the laser light beams, to compensate for astigmatism and collimation changes due to the change in input beam wavelength and adapted to modify the obtained images of the object by electronic means

to maintain alignment of the scan lines of the image at all wavelengths.

3. The laser confocal scanning microscope according to claim 2, said mechanical means comprising a movable astigmatism lens and/or a movable collimating lens placed in said beam path.

4. The laser confocal scanning microscope according to any one of claims 1 to 3, said beam path further including:

a lens (8) positioned between said objective (9) and the second deflector (7) to direct the light beams from said objective (9) onto said second deflector (7), and

at least one spatial filter (14;23) positioned in the return light beam path between said second deflector (7) and said at least one detector (15;26) for effecting confocal imaging,

whereby a frame-scanning movement introduced by said second deflector (7) is adapted to be eliminated as a result of which the return light can be focussed on said at least one spatial filter (14;23).

5. The laser confocal scanning microscope according to any one of claims 1 to 4, wherein a first beam splitter (4) or dichroic mirror (20) is incorporated in the beam path between said acousto-optical deflector (5) and said laser light beam emitting means, for splitting off the return light beam and for directing it to said at least one detector (15), wherein said beam path is constructed such that the return light beam follows the same optical path as the laser light beam up to said first beam splitter (4) or dichroic mirror (20) whereby the line scanning movement introduced by said acousto-optical deflector (5) is eliminated.

6. The laser confocal scanning microscope according to any one of claims 1 to 5, for use in fluorescence microscopy or

other forms of microscopy in which the wavelength of the return beam (19) differs from that of the laser light beams (18) emitted from said laser light beam emitting means, wherein a spatial filter (14) is mounted on an assembly of three piezoelectric crystals and can accordingly be moved in a 3D co-ordinate system, as a result of which the effect of the dispersive nature of the acousto-optical deflector (5) on the return light of a different wavelength, which is deflected through an angle other than the reflected laser light, is eliminated and wherein a correspondingly matched bandpass or cut-off filter (12) is incorporated in the return light beam path to filter out the reflected laser light.

7. The laser confocal scanning microscope according to any one of claims 1 to 6 for use in fluorescence or other forms of microscopy in which the wavelength of the return beam (19) differs from that of the laser light beam (18), wherein a dichroic mirror (20) is incorporated in the beam path between the acousto-optical deflector (5) and the second deflector (7) in order to deflect the return light beam with differing wavelengths downstream of the second deflector and to direct it via an objective (22) and a subsequent spatial filter (23) to a subsequent detector (26), the subsequent spatial filter (23) being a slit filter which forms a line detector with the subsequent detector (26).

8. The laser confocal scanning microscope according to claim 5 or 7, wherein the return light beam is adapted to be divided into a plurality of light beams by means of an additional beam splitter or dichromatic mirror or a plurality of additional beam splitters or dichromatic mirrors inserted into the return beam path after the return beam path is separated from the input beam path by the first beam splitter (4) or dichroic mirror (20), each resulting beam is adapted to be directed to one or more detectors, each detector having a spatial filter, objective, and lens duplicating the single

detector beam path, except that a bandpass or cut-off filter may be different for each detector.

9. The laser confocal scanning microscope according to any one of claims 1 to 8, wherein said electronic control and imaging system is adapted to provide synchronisation of the selected laser light beam wavelength to the flyback or other selected time point in the line scans by applying control signals to a wavelength selection means mounted downstream of said laser light source (1) such that the laser light beams passing through said wavelength selection means on their passage into or through the beam path are controlled such that only the selected wavelength is permitted to pass through the beam path.

10. The laser confocal scanning microscope according to any one of claims 1 to 8, wherein said electronic control and imaging system is adapted to provide synchronisation of the intensity modulation or blanking of the laser light source beam to the flyback or other selected time point in the line scans by applying control signals to an intensity modulation means mounted downstream of said laser light source (1) such that the laser light beams passing through said intensity modulation means on their passage into or through the beam path are controlled such that the intensity of the light beams can be modulated or blanked.

11. The laser confocal scanning microscope according to claim 9, wherein said wavelength selection means comprises an acousto-optical tuneable filter (AOTF).

12. The laser confocal scanning microscope according to claim 10, wherein said intensity modulation means comprises an acousto-optical tuneable filter (AOTF) and/or said acousto-optical deflector (5).

13. The laser confocal scanning microscope according to any one of claims 1 to 12, wherein said electronic control and imaging system is comprised of hard wired logic, a Digital Signal Processor, a microprocessor, a computer or a similar computational device.

14. The laser confocal scanning microscope according to any one of claims 1 to 13, wherein said laser light source (1) includes a multi-line laser, a tuneable laser, and/or an array of lasers emitting at various wavelengths and an optical configuration that provides collinear laser beams.

15. The laser confocal scanning microscope according to any one of claims 1 to 14, wherein said second deflector (7) comprises a mirror galvanometer.

16. The laser confocal scanning microscope according to any one of claims 1 to 15, wherein the light beams are coupled to the beam path by means of a rigid or flexible optical light guide.

17. The laser confocal scanning microscope according to claim 16, wherein the optical light guide is an optical fibre.

18. A method of achieving fast multi-wavelength scanning in an acousto-optical deflector based laser confocal scanning microscope, comprising:

dynamically adjusting drive parameters of said acousto-optical deflector (5) in accordance with a selected wavelength of a laser light beam, to maintain alignment of scan lines of an image of an object at all wavelengths.

19. A method of achieving fast multi-wavelength scanning in an acousto-optical deflector based laser confocal scanning microscope, comprising:

dynamically adjusting an optical path of said an acousto-optical deflector based confocal microscope by mechanical means in accordance with a selected wavelength of a laser light beam, to compensate for astigmatism and collimation changes due to the change in input beam wavelength and modifying detected images of an object by electronic means to maintain alignment of the scan lines of the image at all wavelengths.

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VisiTech International Ltd

Abstract

Fast multi-line laser confocal scanning microscope

According to a first embodiment the invention provides, for achieving fast multi-wavelength scanning in an acousto-optical deflector based laser confocal scanning microscope, dynamically adjusting an optical path of said an acousto-optical deflector based confocal microscope by mechanical means in accordance with a selected wavelength of a laser light beam, to compensate for astigmatism and collimation changes due to the change in input beam wavelength and modifying detected images of an object by electronic means to maintain alignment of the scan lines of the image at all wavelengths. According to a second embodiment the invention provides, for achieving fast multi-wavelength scanning in an acousto-optical deflector based laser confocal scanning microscope, dynamically adjusting drive parameters of the acousto-optical deflector in accordance with the selected wavelength of the input laser light beams, to maintain alignment of the scan lines of the image at all wavelengths.

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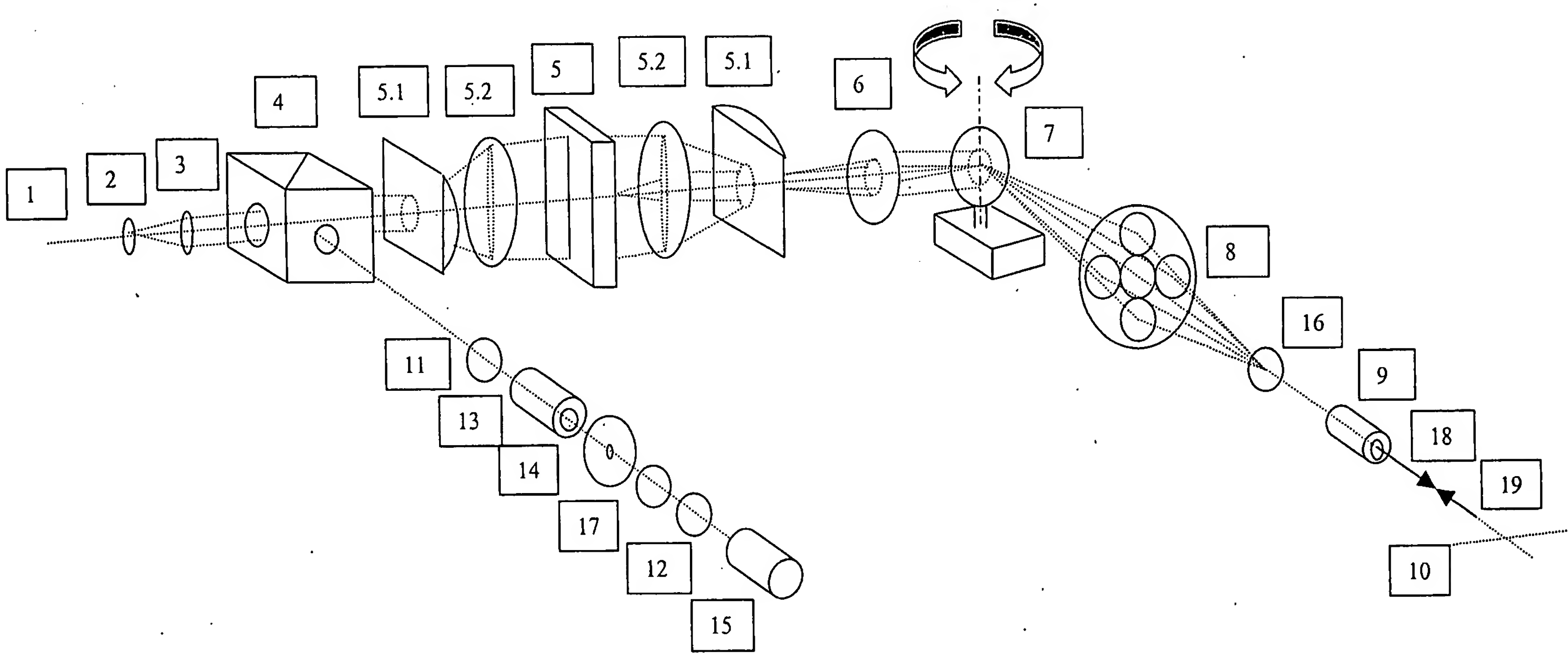

Figure 1

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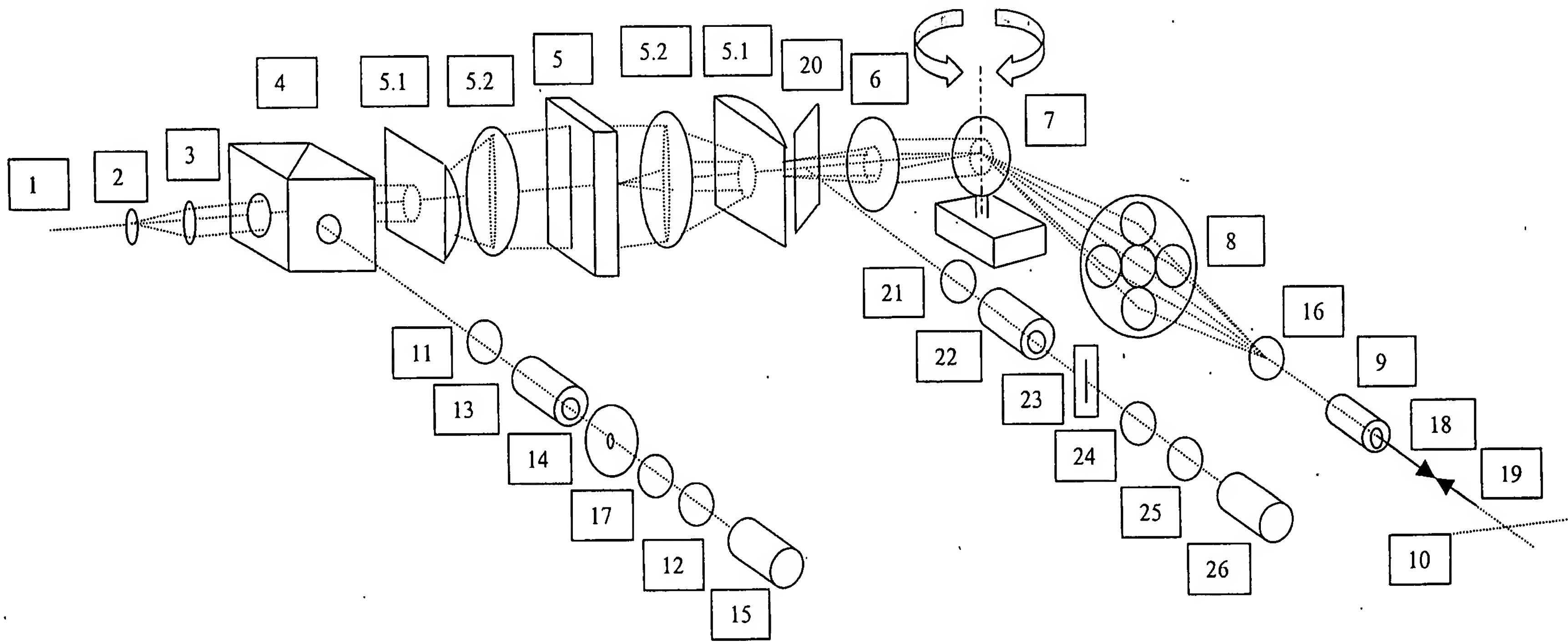
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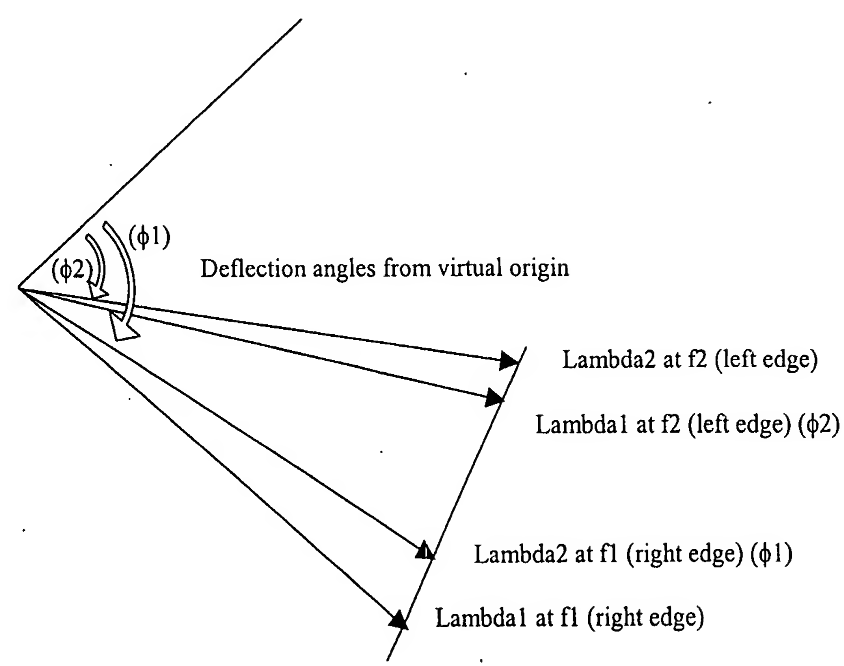
Figure 2

Uncorrected left and right deflection positions		
wavelength	**Left edge (ϕ)**	**Right edge (ϕ)**
Lambda2	(ϕ2)* lambda2/lambda1	(ϕ1)
LambdaN	(ϕ2)* lambdaN/lambda1	(ϕ1)* lambdaN/lambda2
Lambda1	(ϕ2)	(ϕ1)* lambda1/lambda2



Note that angular deflection is greatly exaggerated for clarity in the diagram.

Figure 3